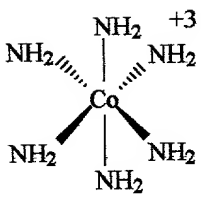
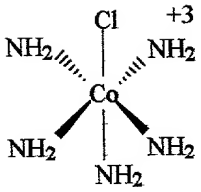
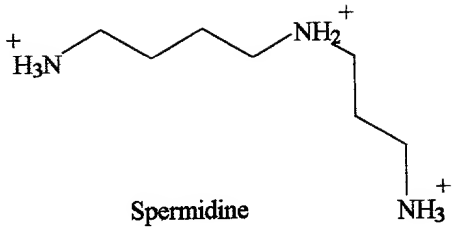
Fig. 1



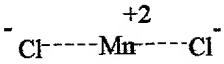
Hexammine Cobalt



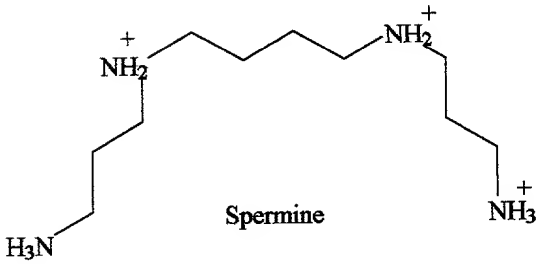
Chloropentammine Cobalt



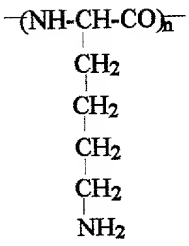
Spermidine



Manganese Chloride



Spermine



(D or L) Polylysine

Figure 1. Structures of common compaction agents

Fig. 2

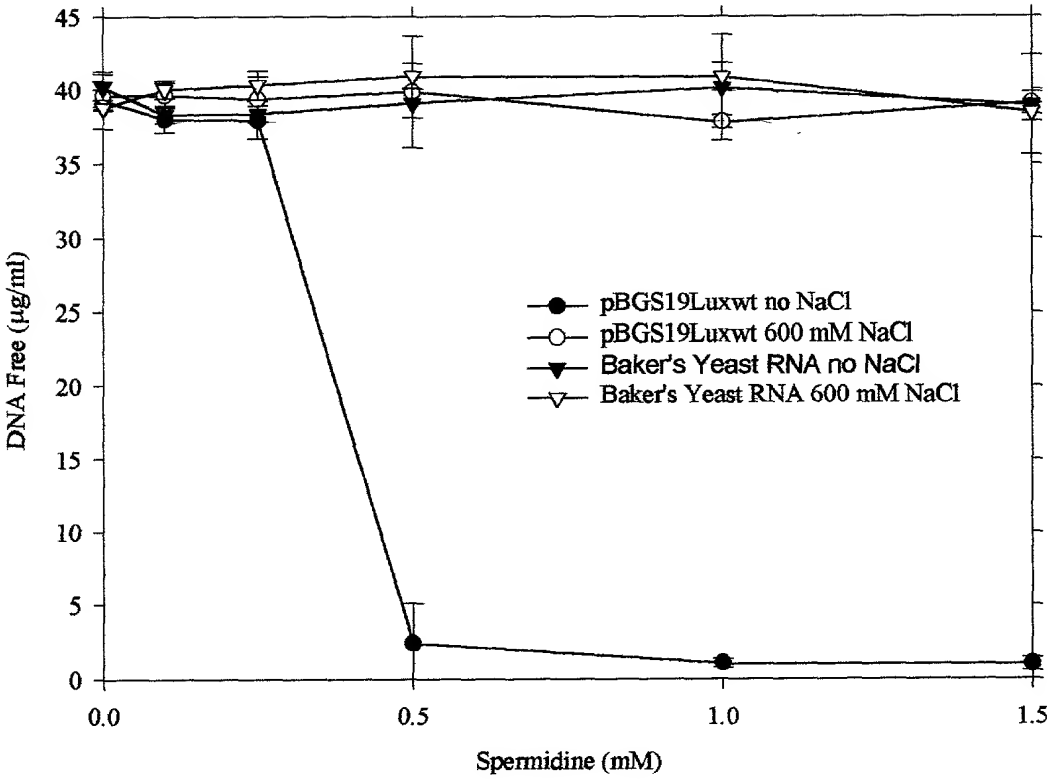


Figure 2. Precipitation by spermidine of 40 µg/ml pBGS19Luxwt or Baker's yeast RNA in 10 mM Tris buffer at pH 8.0 with and without 600 mM NaCl. Error bars are +/- one standard deviation.

Fig. 3

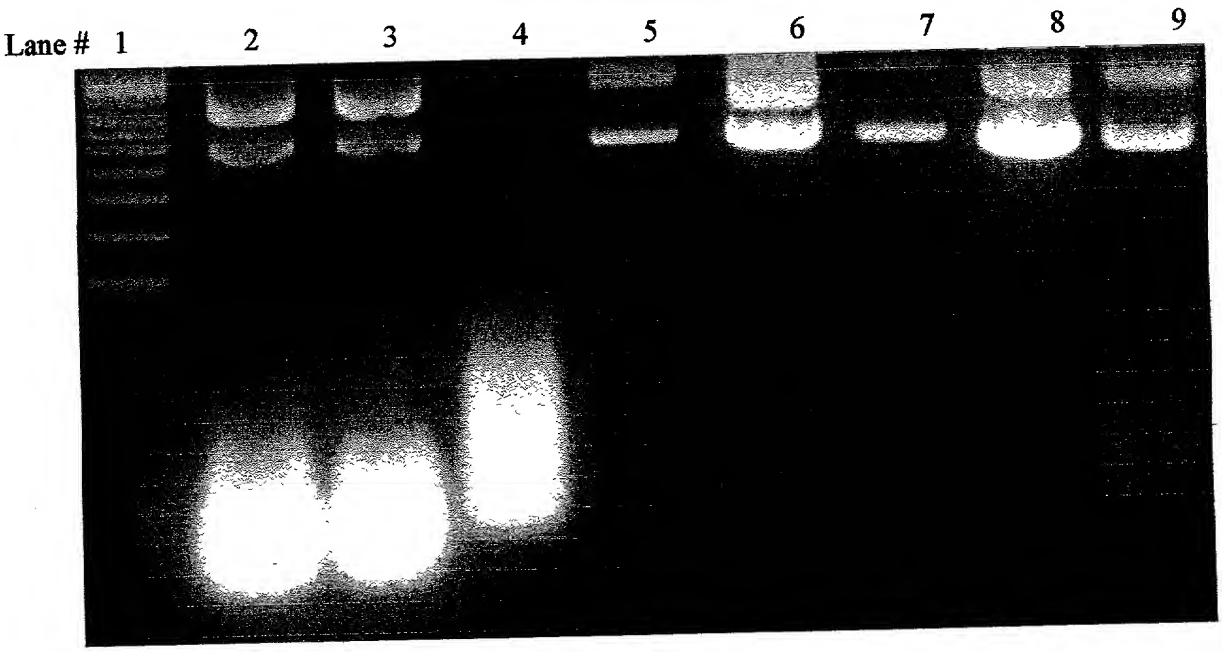


Figure 3. 1% agarose gel that traces the large-scale purification of pBGS19luxwt. Lane 1 is a supercoiled plasmid ladder from Gibco; Lane 2 is the preparation after Celite filtration, isopropanol precipitation, and resuspension; Lane 3 is the supernatant after LiCl precipitation; Lane 4 is the supernatant of the compaction precipitation; Lane 5 is the resuspended pellet of the compaction precipitation; Lane 6 is a 10X loading of the material in Lane 5; Lane 7 is after a Q sepharose anion exchange column (Fig. 5, bottom, Peak 5); Lane 8 is a 10X loading of Lane 7 and Lane 9 is pBGS19Luxwt plasmid DNA separated using the mini-prep procedure.

Fig. 4

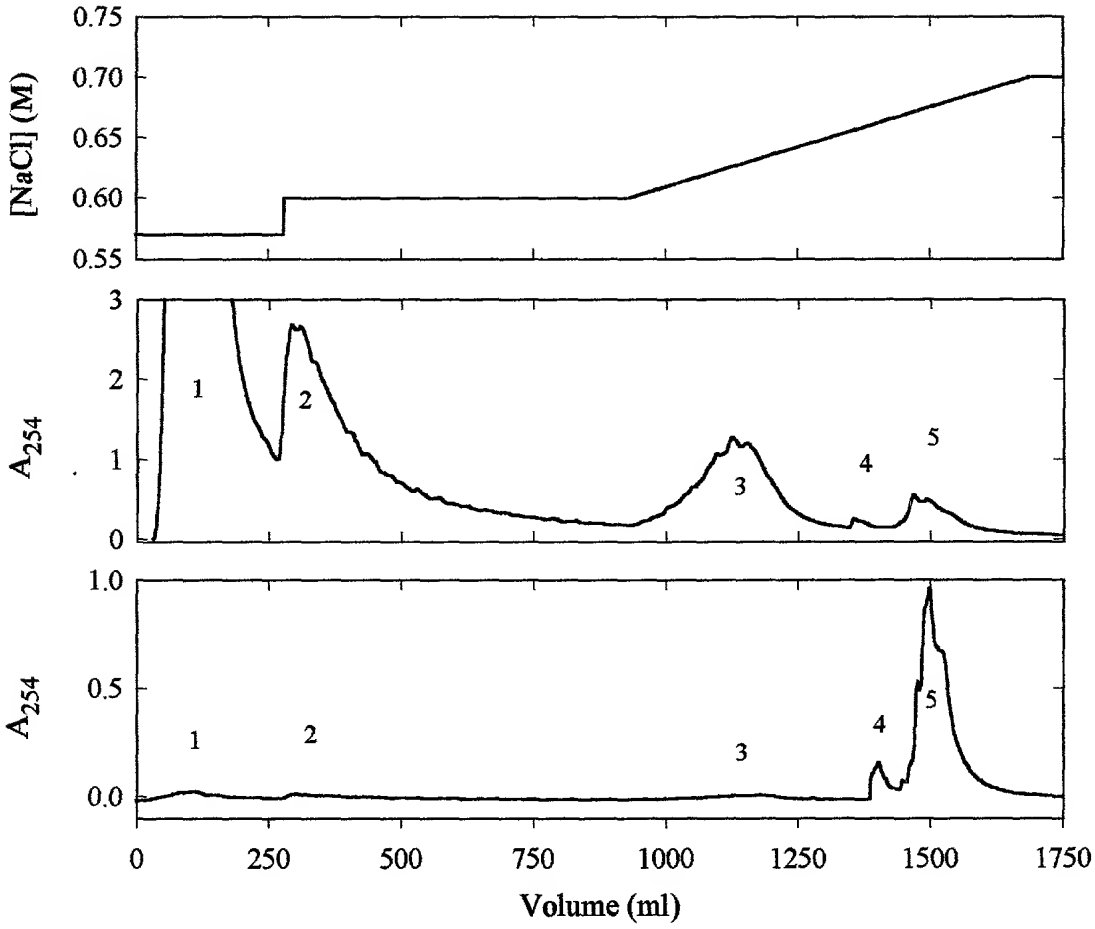


Figure 4. FPLC anion-exchange separation of pBGS19Luxwt of an alkaline lysate after isopropanol and LiCl precipitation. Top: NaCl gradient; Middle: with no previous compaction precipitation step; Bottom: identical separation after a previous compaction precipitation step.

Fig. 5

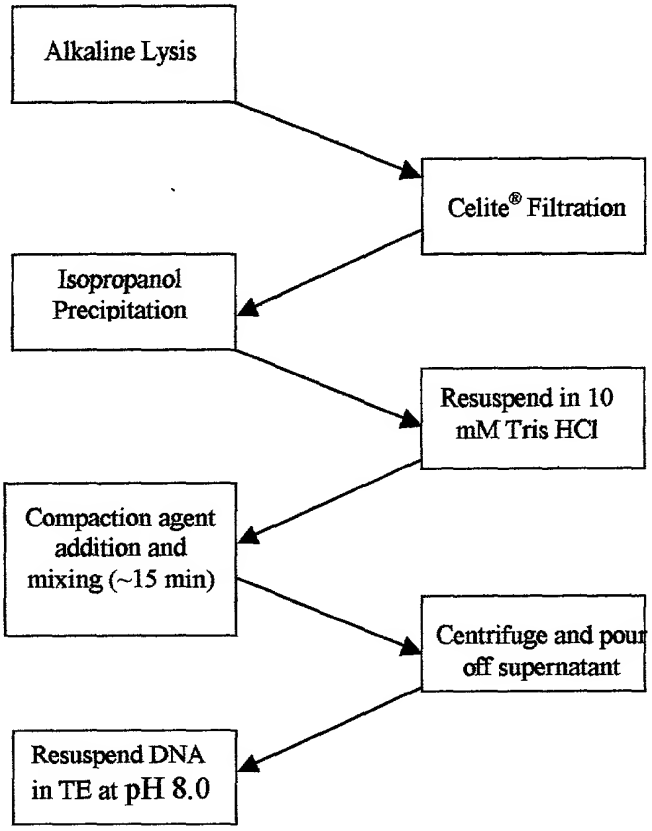


Figure 5. Summary of a selective precipitation-based noncolumn DNA purification as detailed in example 1.

Fig. 6

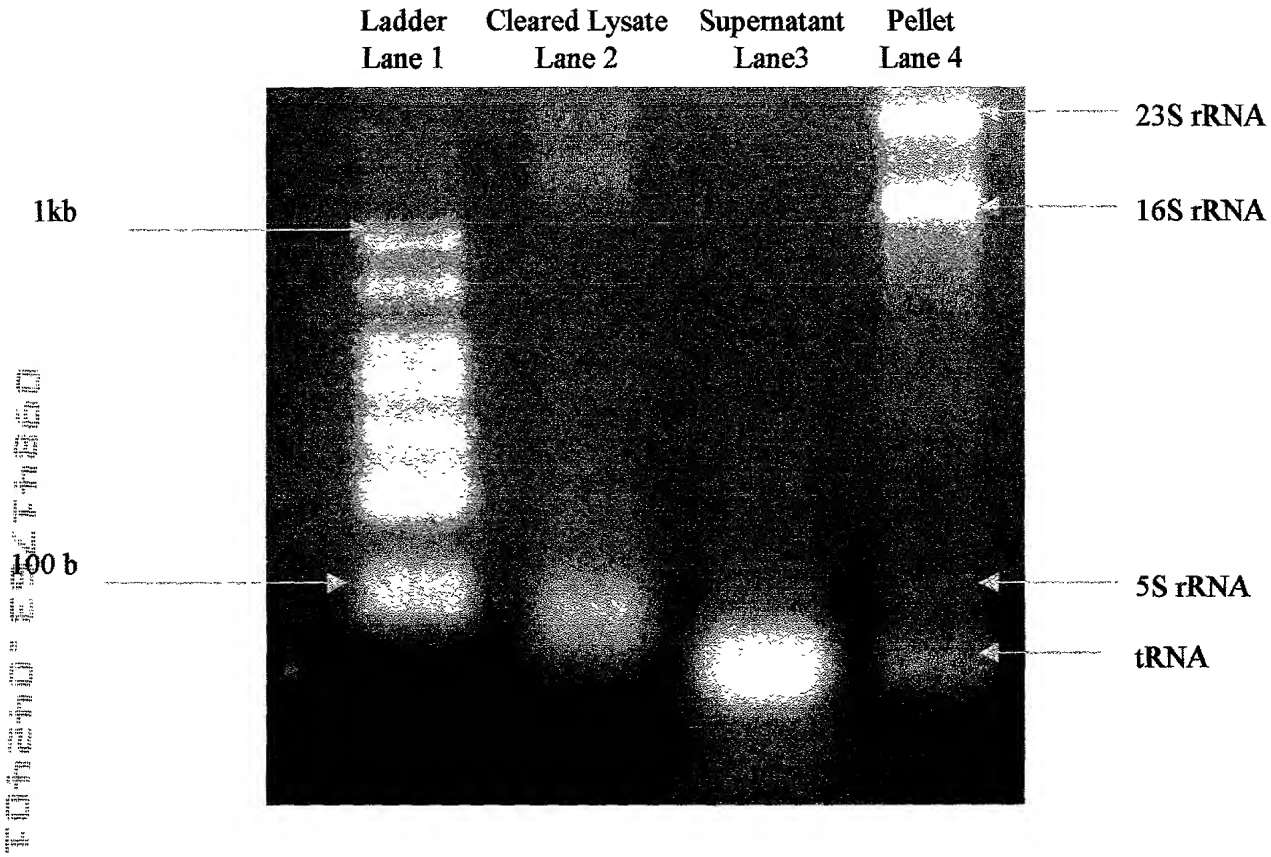


Figure 6. 3% biogel (from Bio101 Inc.) of V. proteolyticus RNA purified by Example 9. Lane 1 is the Ambion RNA Century Plus Size Markers; Lane 2 is the lysate after BPER addition, spermidine addition and centrifugation; Lane 3 is the supernatant of the 4 mM hexammine cobalt precipitation; and Lane 4 is the RNA pelleted in the hexammine cobalt precipitation but before any column separation.

Fig. 7

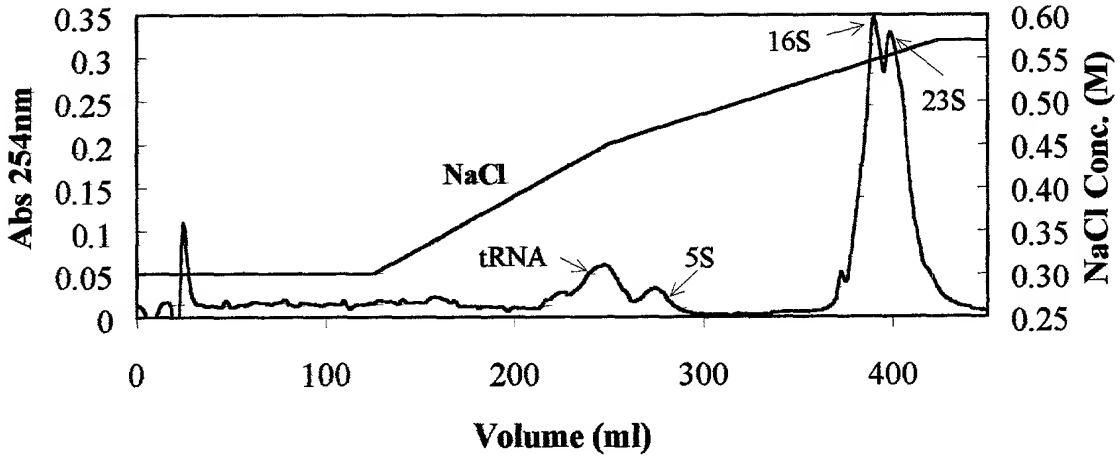


Figure 7. FPLC chromatogram of V. proteolyticus RNA on and 25 ml high performance Q Sepharose anion-exchange column (Pharmacia). The gradient was ran over 12 column volumes from 0.30 M NaCl to 0.57 M NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9.

Fig. 8

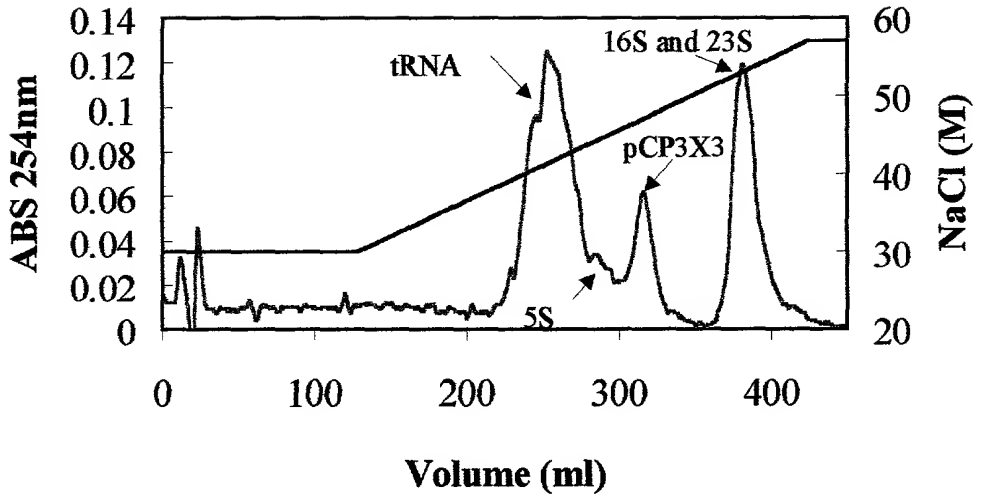


Figure 8. FPLC chromatogram of pCP3X3 aRNA containing *E. coli* strain JM109 on and 25 ml high performance Q Sepharose anion-exchange column (Pharmacia). The gradient was ran over 12 column volumes from 0.37 M NaCl to 0.57 M NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9.

Fig. 9

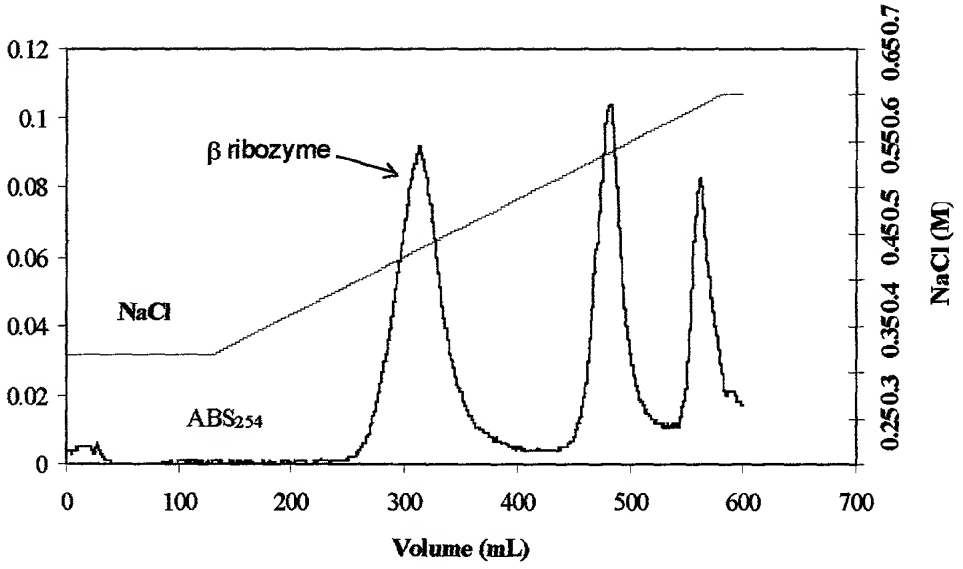
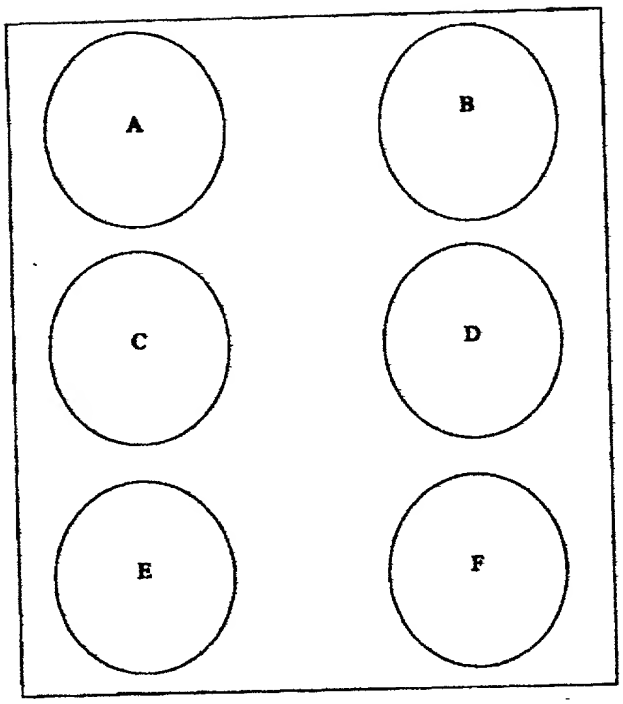


Figure 9. FPLC chromatogram of selective precipitation purified β ribozyme on and 25 ml high performance Q Sepharose anion-exchange column (Pharmacia). The gradient was ran over 12 column volumes from 0.37 M NaCl to 0.7 M NaCl in a column buffer of 10 mM bis-tris propane and 2 mM EDTA at pH 6.9.

Drawing 10B



Description of Drawing 10B

RNA isolation kit based on a hexammine cobalt precipitation process.

A. Lysis/DNA precipitation solution (e.g. 50% HPER with 2.5 mM Spermidine or 1% Brij 58 with 2.5 mM spermidine in 10 mM bis tris propane at pH 7). This may also be extended for use with plant cells and other eukaryotic cells with the possibilities of homogenization, other lysis solutions, and breaking the lysis from the spermidine DNA removal. Thus, there would be a lysis solution and a separate DNA precipitation solution.
B. Hexammine cobalt precipitation solution (e.g. 7 mM hexammine cobalt for a total RNA precipitation or 4 mM hexammine cobalt in 10 mM bis tris propane at pH 6.9 for a high molecular weight precipitation).
C. Optional: a second hexammine cobalt precipitation solution to bring down low molecular weight RNA not precipitated when solution B was used (e.g. 20 mM hexammine cobalt in 10 mM bis tris propane at pH 6.9).
D. Stripping solution (e.g. 50% isopropyl alcohol[a] with 3 M Urea, 300 mM NaCl, 25 mM EDTA).
E. 70% EtOH wash[a,#]
F. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).[#]

[a]User may have to add enough alcohol to bring the solution to the proper percentage of alcohol.

[#]Optional kit components that could be provided by end user.

Drawing 10A

Description of Drawing 10A

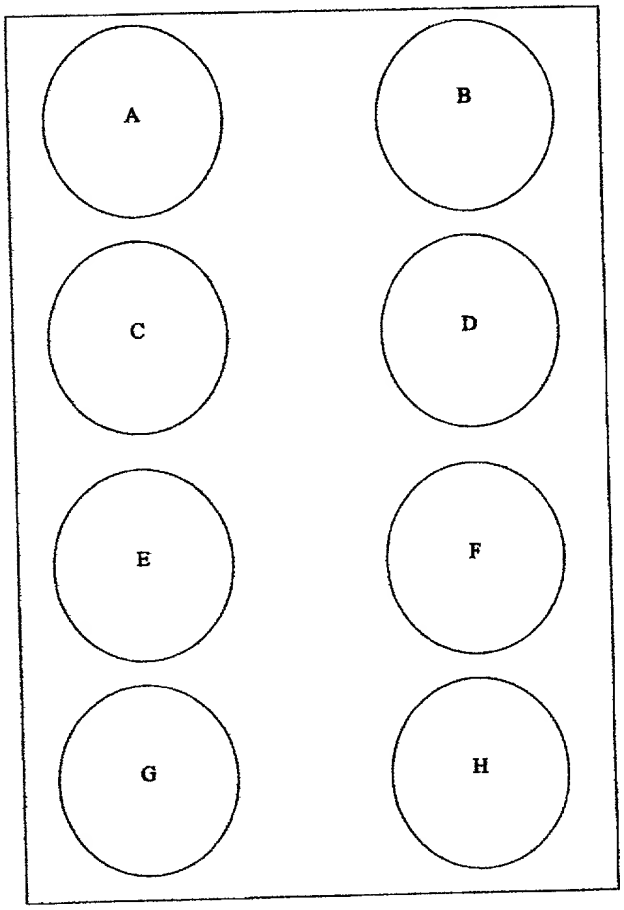
Plasmid DNA separation kit based on compaction agent precipitation technology. In the box a total of 8 solutions should be include two of which are optional. Solutions will include the 3 common alkaline lysis solutions, a low ionic strength resuspension buffer, a compaction agent precipitation solution, and a stripping solution, and optionally a 70% ethanol wash solution and a final resuspension solution.

Description of each bottle are as follows:

A. Alkaline lysis solution I (e.g. 25 mM Tris and 10 mM EDTA at pH 8.0)
B. Alkaline lysis solution II (e.g. 1% Sodium Dodecyl Sulfate (SDS) 0.2 N NaOH)
C. Alkaline lysis solution III (e.g. 3 M KAc at pH 5.5)
D. Resuspension solution (e.g. 10 mM Tris at pH 8.0)
E. Compaction agent precipitation solution (e.g. 2 mM Spermidine 3HCl and 10 mM Tris at pH 8.0_
F. Compaction agent stripping solution (e.g. 50% EtOH, 300 mM NaCl, 12.5 mM EDTA[a])
G. 70% EtOH wash[a,#]
H. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).[#]

[a]User may have to add enough EtOH to bring the solution to the proper percentage of EtOH.
[#]Optional kit components that could be provided by end user.

Also, solutions D and E can be combined to form a resuspension/compaction agent precipitation solution.



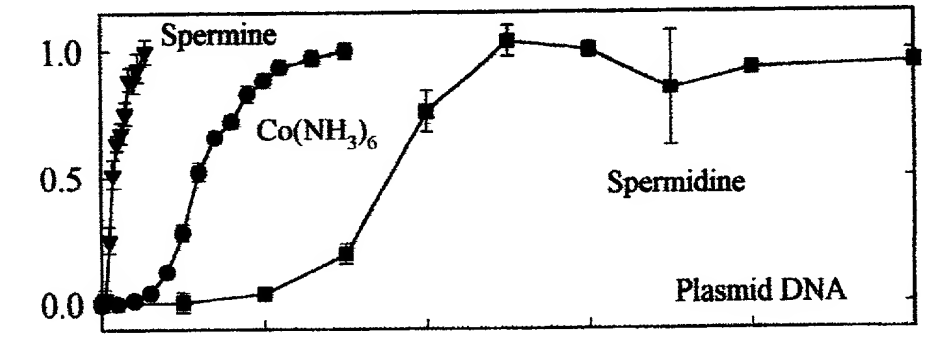


Fig. 11

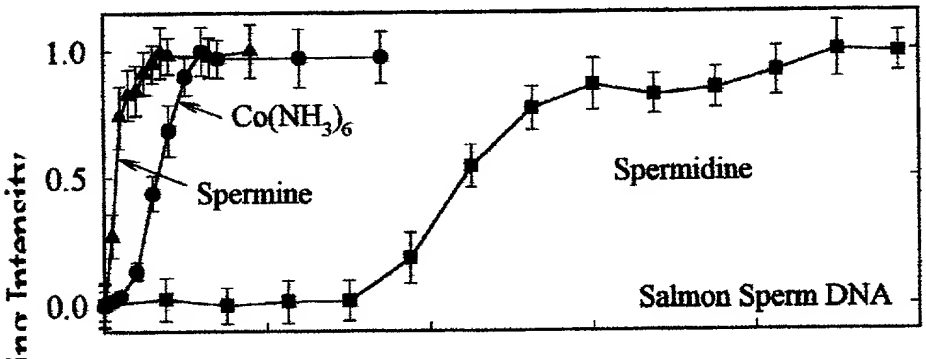


Fig. 12

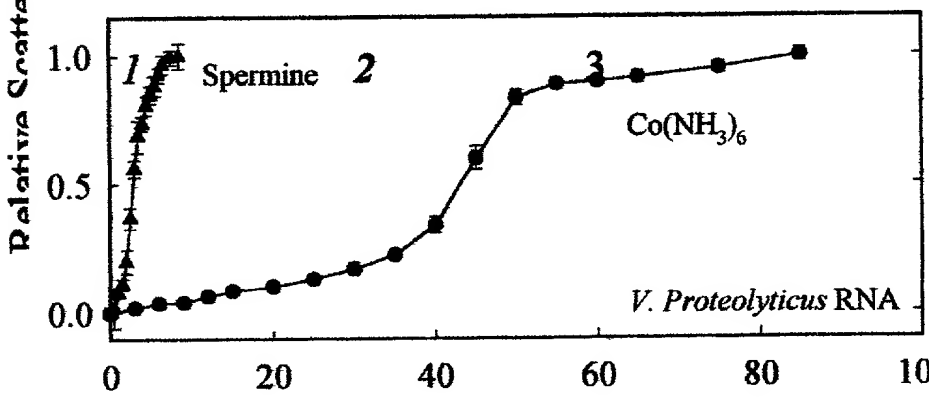


Fig. 13

Docket 009MUS

Charge Equivalents of Compaction Agent

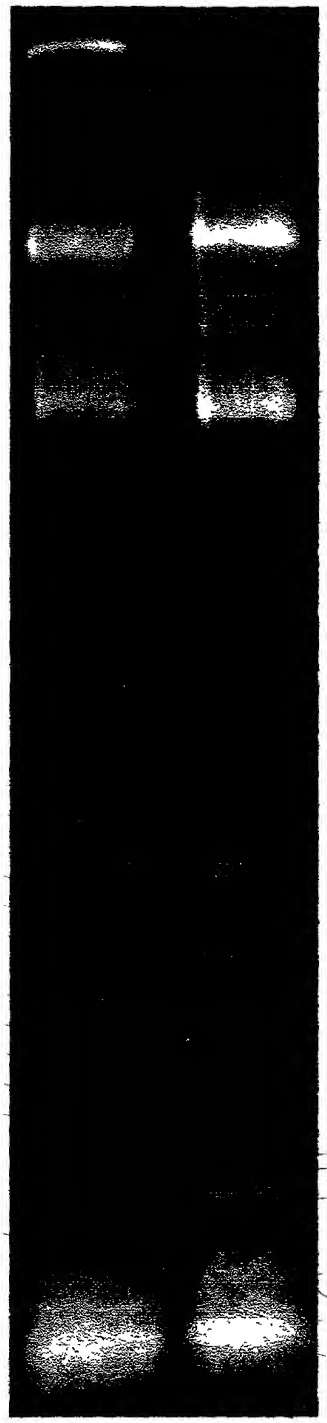


Fig. 14

Fi

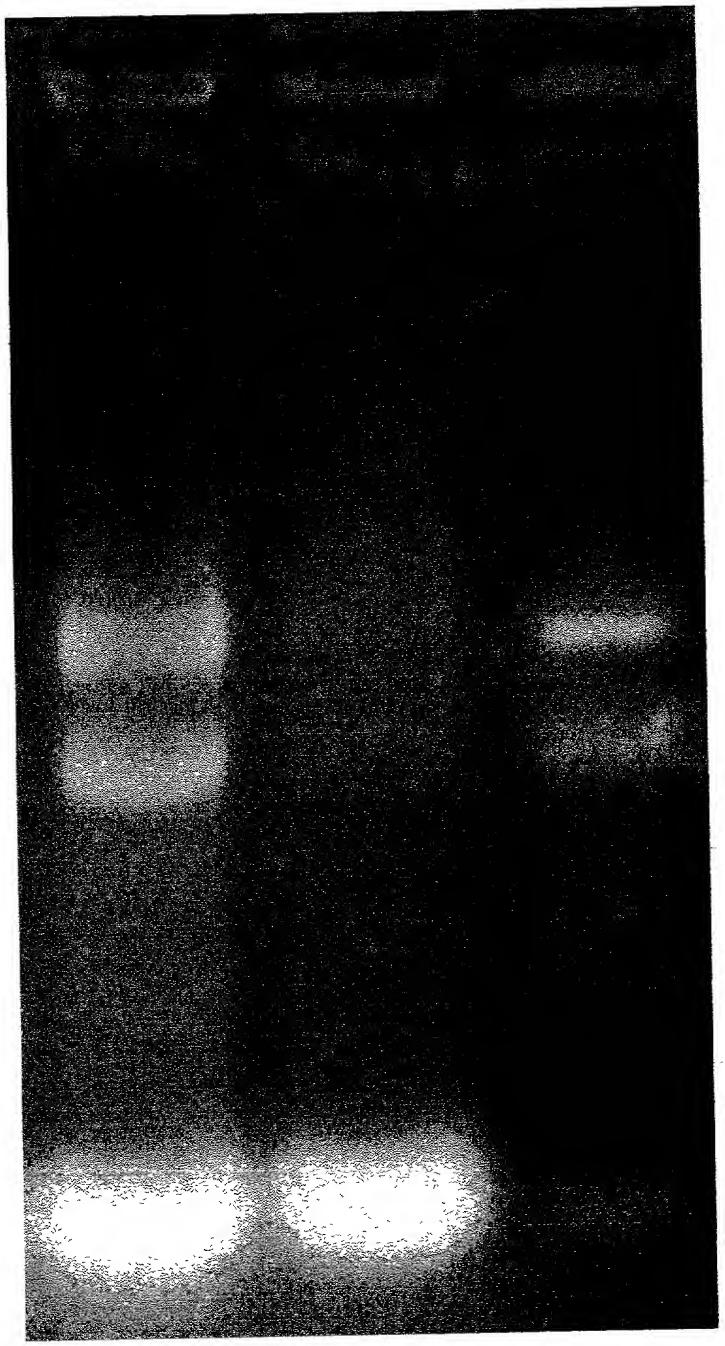


FIG. 15

5

FIG.16

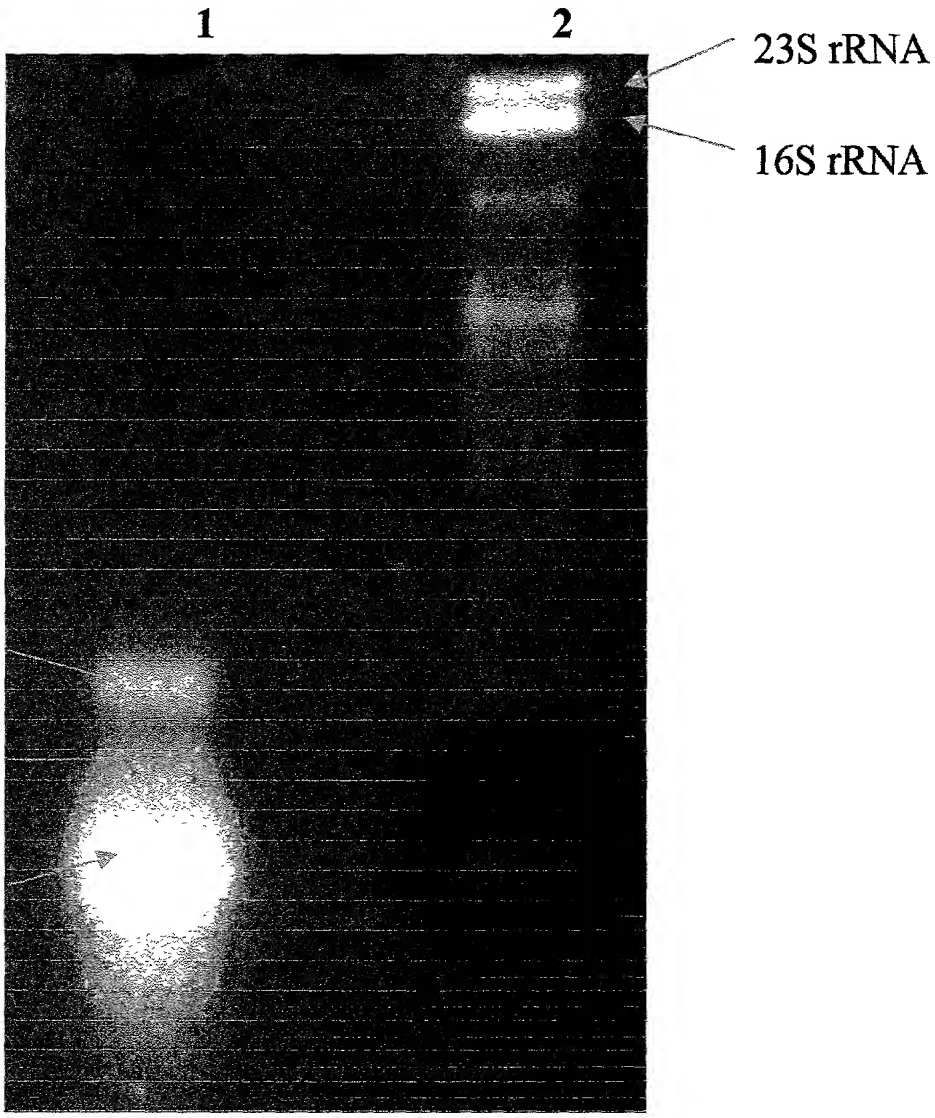


FIG.17

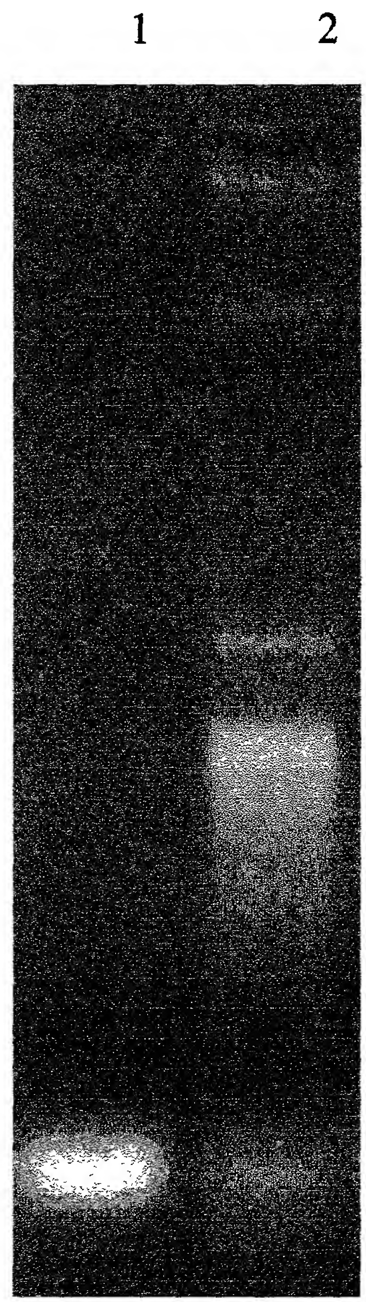
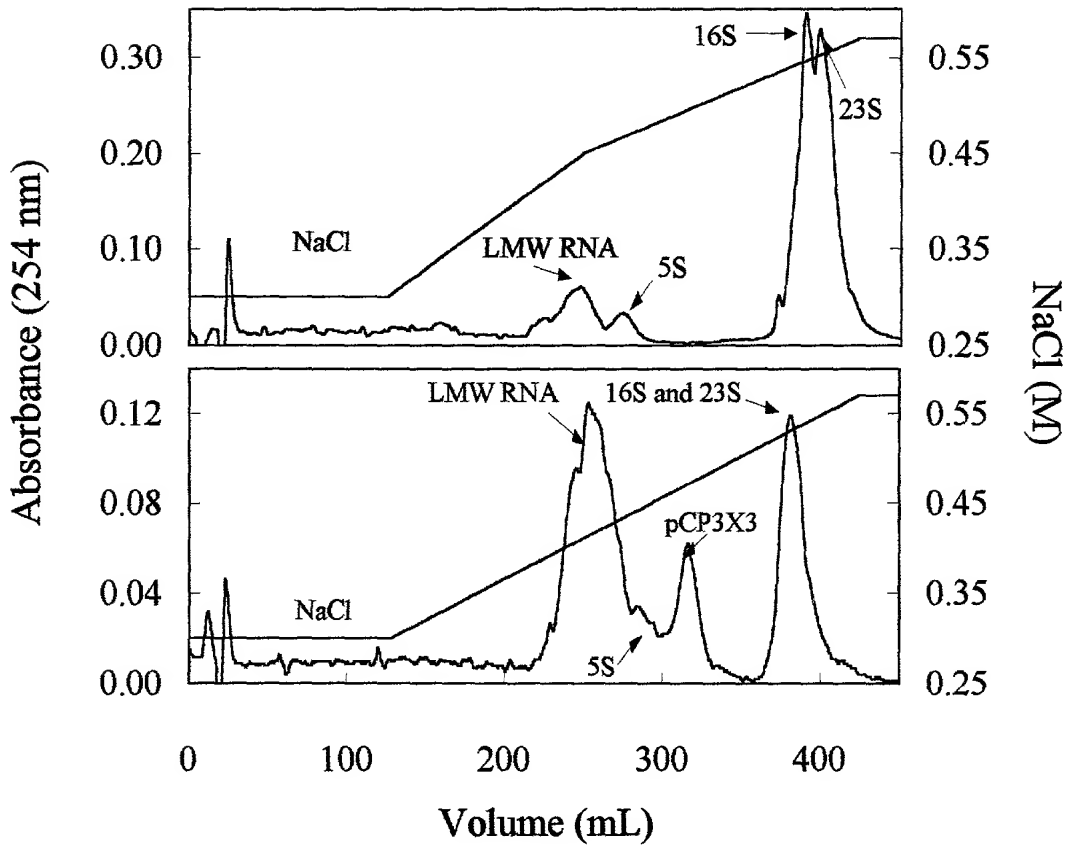


FIG.18


16S
23S
NaCl
LMW RNA
5S
LMW RNA
16S and 23S
pCP3X3
NaCl
5S
0.30
0.20
0.10
0.00
0.12
0.08
0.04
0.00
0.55
0.45
0.35
0.25
0.55
0.45
0.35
0.25
0
100
200
300
400
Absorbance (254 nm)
NaCl (M)
Volume (mL)